Activity and structural changes of Mashroom Tyrosinase after its modification by Woodwaed Reagent K

CBC13thP2T7

Saeed Emami[1*], Nematollah Gheibi[2]

[1]Department of Biophysics, Azad University Science and Research branch, Tehran, Iran
[2]Department of Biophysics and Biotechnology, Qazvin University of Medical Science, Qazvin, Iran
*Abstract presenter: Sayid.emami@gmail.com

Mashroom Tyrosinase (E.C. 1.14.18.1) known as polyphenol oxidase, is a multifunctional, glycosylated, and copper containing enzyme from the oxidase superfamily widely distributed in microorganisms, plants, and animals. It has a key role in melanin biosynthesis catalyzing two divergent reactions: The hydroxylation of monophenols (cresolase or monophenolase activity) and the oxidation of o-diphenols (catecholase or diphenolase activity) into reactive o-quinones. Modification of residues in protein sequences is a good method for understanding of their structure function relationship. The polar amino acids such as glutamate, aspartate, lysine, arginine, histidine, serine, tyrosine, methionine and tryptophan can be modified by chemical reagents. In this work, kinetic and structure of mushroom tyrosinase were assessed in its native and modified forms. The modification occurred on glutamate and aspartate residues of the enzyme by Woodward reagent k. The kinetic activity of native and modified MT obtained through catecolase reaction of enzyme by L-Dopa as substrate. The kinetic data were analysed by double reciprocal Lineweaver-Burk plots and values of V_{max} and K_m parameters obtained for the enzyme with different concentrations of the modifier (0, 0.5, 1, 5 and 10 mM). The magnitudes of V_{max} and K_m in the previous mentioned of modifier concentrations were determined 9.6, 9.2, 8.8, 6.4 and 4.8 $(mM/min)^{-1}$ and 2.3, 2.5, 2.6, 3.5 and 4.7 mM respectively. Thus, gradually decreasing in V_{max} and increasing in K_m values revealed the lower activity of modified enzyme and reduction in the MT substrate affinity in comparison with its native form. In line with kinetic data the structural analysis of modified enzyme by circular dichroism and intrinsic fluorescence showed its instability in comparison with its native form.

Key words: Mashroom Tyrosinase, Woodward reagent k, Modification, Kinetic, Structure